

Abstract

The present invention relates to *in vitro* cultured skin substitutes, and in particular to *in vitro* cultured skin substitutes that have improved barrier function. In some embodiments, improved barrier function is a result of improved culture conditions, while
5 in other embodiments, improved barrier function results from genetic modification of keratinocytes. Improved culture conditions to improve barrier function include organotypic culture in the presence of linoleic acid and/or linoleic acid at about 75% humidity. Suitable genetic modifications for improving barrier function includes transfection with a DNA construct capable of expressing GKLF.